DATA DEFRAGMENTATION – OVERCOMING ANALYTICAL ERRORS DUE TO ION-SOURCE FRAGMENTATION OF CO-ELUTING CONGENERS

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Overview and Introduction:

- Fragment ions formed in a mass-spectrometer's ion source due to losses of Cl_x (or Br_x) can give rise to interference peaks within the retention time window of specific target compounds. For example, Hexa-CB 146 can lose a single chlorine to form a peak at the same retention time as Penta-CB 105 and has ions that are not resolved in mass (see figure 1) using standard HRMS conditions, i.e. 10,000 resolution (EPA Method 1668A[1], SPB-Octyl column).
- These interferences can have a significant effect on the identification and reported concentrations of certain compounds[2] – in some instances accounting for their entire value – and yet the errors may go unnoticed as resultant peaks often still meet the method's key identification criteria of isotope ratio and relative retention time. In other cases, such as when the interfering fragment is due to the loss of Cl_2, the affected peak may be out-of-ratio thus giving a false negative, or an EMPC result.
- For PCBs analyzed using 1668A or similar methods, several WHO specific and EU marker compounds are subject to this problem; even small interferences on certain TEF bearing compounds can significantly alter a sample's TEQ. Although 1668A (and similarly EPA Method 1614 for polybrominated diphenyl ethers) references the potential problem of fragment peaks due to the loss of one or more chlorine (or bromine) atoms, there are no specific guidelines on how to measure, or even estimate, the significance of such peak. Method 680[3], for low-resolution analysis, uses a strategy of monitoring certain fragment ions and applying area corrections to co-eluting peaks. However this approach is not directly applicable to high-resolution analysis due to the differences between the exact masses of the fragment ions and those of the target.
- We explore the use of monitoring certain fragment ions, but at offset masses that take into account the aforementioned differences. From these data, the effect on the various target masses can be computed and any changes in instrument resolution, or other conditions, are inherently corrected for. These derived fragment data can then be subtracted from the raw data prior to peak detection thereby providing a true correction on a scan-by-scan basis.

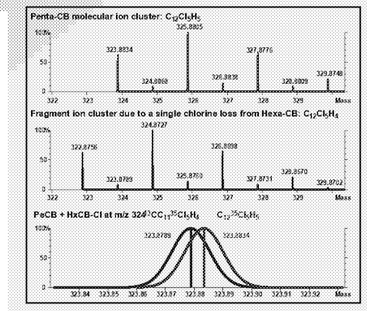

Figure 1. Theoretical isotope cluster patterns of the molecular ion for Penta-CB (red trace) and the fragment due to the loss of a single Chlorine from Hexa-CB (blue trace). Both traces are overlaid in the lower box to show the ~14 ppm separation at m/z 324

Target PCB	Homolog group	Precursor: loss of Cl	Precursor: loss of Cl_2	TEF WHO-2005	Marker (EU)
PCB-28	Tri	PCB-45	-	-	Y
PCB-77	Tetra	PCB-111	-	0.0001	
PCB-81	Tetra	PCB-115	-	0.0003	
PCB-101	Penta	PCB-152	-	-	Y
PCB-105	Penta	PCB-146	-	0.00003	
PCB-114	Penta	PCB-133	PCB-188	0.00003	
PCB-118	Penta	PCB-132	-	0.00003	Y
PCB-123	Penta	PCB-131	-	0.00003	
PCB-126	Penta	PCB-128/166	PCB-175	0.1	
PCB-167	Hexa	PCB-181	-	0.00003	
PCB-169	Hexa	PCB-190	PCB-198/199	0.03	

Table 1. The WHO and EU Marker PCBs affected by fragmentation during analysis using the SPB-Octyl GC column, and the precursors (sources) of those fragments

Materials and Methods

To examine this effect, we have evaluated data from several calibration and performance standards and some field samples, including one from an international 'round-robin' food study[4]. To date, we have focussed our fragmentation studies on PCBs because the principal method for the analysis of all 209 possible congeners, Method 1668A, uses the SPB-Octyl as its preferred analytical column; although this column achieves the method's primary separation requirements, the resultant chromatography has several overlapping homolog groups that give rise to this problem. In contrast, although PCDD/Fs may be similarly affected when using certain columns such as the 2331, routine analyses based on Methods 8290 or 1613 using relatively non-polar columns with a 5% phenyl phase—the "5" and "5ms" types—are unaffected as, with one minor exception, each of the Tetra-Octa homolog groups are resolved into separate time windows

The degree of fragmentation that occurs is a complex function dependent on many variables including: the structure of the molecule(s) undergoing fragmentation, ionization parameters such as electron energy and source temperature, and the gas pressure in the ion source at that instance in time. Since the former could be a mixture of isomers of unknown distribution, and the latter also unknown due to its sample dependence (often including substantial quantities of matrix interferences that were not completely removed during clean-up), knowledge of the precursor ion intensities can only provide a very approximate indication of any effect on the target analytes. Furthermore, since the exact masses of the ions in the fragment's isotope cluster are slightly different from those of the target with which they interfere, the instrument's actual mass resolution also needs to be taken into account.

GC-HRMS analyses of the PCBs were performed using a Waters/Micromass AutoSpec-Ultima mass-spectrometer operating at ~12,000 resolution using EI ionization at 34 eV with a source temperature of 260 °C. Sample introduction was via an Agilent 6890 GC fitted with a CTC GC-PAL auto-sampler and a Supelco SPB-Octyl GC column (30 m x 0.25 mm x 0.25 µm). Helium was used as the carrier gas with the injector operating in constant-flow mode. The SIR data were acquired using MassLynx software and then transferred to our own UltraTrace-Pro (UTP) software for processing and display.

Results and Discussion

Initial observations

An analysis of data from a calibration standard containing all 209 PCB isomers revealed that, within a 5 second search window, there were 44 possible cases of congeners occurring with higher chlorinated co-eluting peaks. Of these, 40 corresponded to a single chlorine difference, and the remaining 4 to Cl_2. Interestingly, in each case where a congener was a candidate for fragment interference due to the loss of Cl_2, it was also affected by another fragment due to a single Cl loss; 3 of these 4 instances corresponded to a WHO specific congener being affected: PCBs 114, 126 and 169 (the other is PCB-38). The WHO PCBs affected only by a single Cl loss were: 77, 81, 105,118, 123 and 167. The means the only unaffected specific PCBs (using this chromatography) were 156, 157 and 189.

The ±5 second window was chosen based on the typical (Penta-CB) GC peak width being approximately 10 seconds, and the allowed relative retention time window defined by the method for unlabelled peak identification being typically 6 to 10 seconds. Table 1 shows the WHO list and marker PCBs that are subject to effects of fragment peaks and the corresponding precursors. The marker, or indicator, PCBs comprise the following congeners: 28, 52, 101, 118, 138, 153 and 180; these are often used in studies involving food or feed samples, particularly within the EU. PCB-118 is a member of the WHO list and is also a marker PCB.

Mass calculations

In this paper we will focus on the effects relating to the Penta-CBs, although the principles employed apply similarly to the other homolog groups. The M and M+2 ions monitored for Penta-CB are 323.8834 ($C_{12}H_5{}^{35}Cl_5$) and 325.8804 ($C_{12}H_5{}^{35}Cl_4{}^{37}Cl$); the M and M+2 fragment masses formed when a Hexa-CB loses a chlorine are 322.8756 ($C_{12}H_4{}^{35}Cl_5$) and 324.8727 ($C_{12}H_4{}^{35}Cl_4{}^{37}Cl$). At first, this would not seem to raise any concerns, however we also need to consider the naturally occurring ^{13}C ions present in the fragment's isotope cluster. The M+1 and M+3 ions have masses 323.8789 ($^{13}CC_{11}H_4{}^{35}Cl_5$) and 325.8760 ($^{13}CC_{11}H_4{}^{35}Cl_4{}^{37}Cl$), and are only ~14 ppm lower in mass than the M and M+2 ions of Penta-CB; because these are not resolved, any fragment ions present would contribute to our measurements.

A strategy for measurement and correction

Monitoring the interfering fragments (323.8789 and 325.8760) is not viable since they are equally affected by the Penta-CBs that we are trying to measure. We could instead monitor the base peak of the fragment cluster (322.8756) since that is unaffected by the Penta-CBs, and then use the theoretical isotope cluster pattern to determine the contribution level from the M+1 and M+3 ions; the relative intensities of these isotope peaks are invariant with source ionization or other parameters of the HRMS system. However, as noted above, these masses are 14 ppm away from our target masses—this means their actual effect would also be a function of the instrument's resolution (and peak shape) at that exact time. This is not easy to determine and is prone to error.

The solution we have found, and have implemented in our software, is to measure the intensity of the fragment's base peak at a mass that is offset by +14 ppm from its exact mass. Now when we calculate the intensities of the M+1 and M+3 ions from the theoretical distribution, we obtain each ion's true contribution to those of the target. We can then apply a corresponding correction to the raw data. In our Penta-CB example, we would therefore monitor mass 322.8800, this being 322.8756 + 14 ppm (actually 13.7 ppm). Any variations in resolution or peak shape—such as skew or kurtosis—are now intrinsically adjusted for, since the same effect applies to all peaks over this small mass range. Additional corrections were also made for the loss of Cl_2 (including its effect on the ion we monitor for the single Cl loss). It is important to realize that these corrections are calculated, and applied, on a scan-by-scan basis. When the user activates the "defrag" option, the data is immediately transformed, and further processing can continue with the fragments now removed. Changes to the instrument's tuning or chromatographic conditions have no bearing on the operation of this process.

Results

Figures 2 and 3 show data from the round-robin sample before and after de-fragmentation. The distortion on PCB-105 due to the fragment from PCB-146 can be clearly seen in the original, but is removed in the de-fragmented data. Perhaps less obvious at first sight, is the peak broadening present on PCB-114 due to PCB-133's fragment. In particular the response of PCB-114 in the de-fragmented data was approximately 55% of the original. To validate the quantification of this compound we compared the results with those of the same sample analyzed using a J&W DB-1 column (30 m x 0.25 mm x 0.25 µm) where the 114 peak was effectively resolved from any interferences. The original SPB-Octyl data gave a concentration for PCB-114 that was ~71% higher than the DB-1 result. The de-fragmented data, however, was in very good agreement with a difference of ~6%.

Follow-up

Since writing the original short paper, we have now obtained the mean consensus values from the round-robin study: compared to these, our original value for PCB-105 differed by ~11%; the de-fragmented value would have reduced that to ~4%. Due to the obvious interference affecting PCB-114 (on the SPB-Octyl) we reported a value from a confirmation column – this differed by ~16%; the non-reported value would have been in error by approximately a factor of 2 (!) – however, the de-fragmented data gave a result that differed by just 6% from the consensus mean.

Future studies

It appears that this automated method for removing the effects of fragment peaks is a viable proposition for HRMS data, and could become a valuable tool for production samples, we therefore intend to continue our R&D efforts by comparing data from SRM and other reference samples.

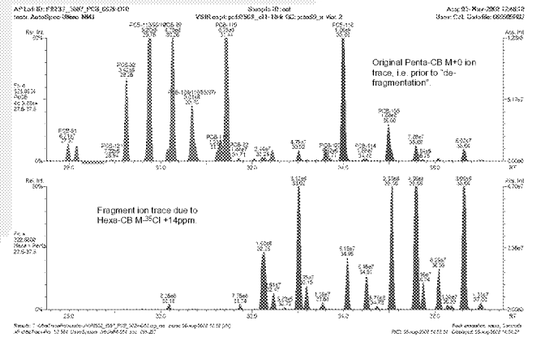

Figure 2. The upper trace shows the original Penta-CB data (m/z 324) for the Eel sample. The lower trace shows the monitored ion for the single chlorine loss from the Hexa-CB

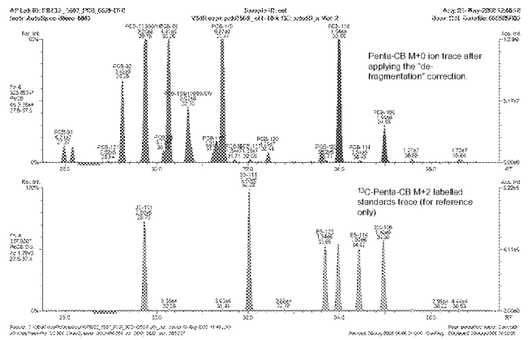

Figure 3. The upper trace shows the de-fragmented data for Penta-CB data (m/z 324). The lower trace shows the Penta ^{13}C-labelled standards' trace.

References.

1. Method 1668 (Rev. A), Telliard W., et al., US Environmental Protection Agency, Dec. 1999
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4. Interlaboratory Comparison on Dioxins in Food, Folkehelseinstituttet (Norwegian Institute of Public Health), 2008